SEXUAL ACTIVITIES OF THE SQUID, LOLIGO PEALII (LES.)

I. COPULATION, EGG-LAYING AND FERTILIZATION

GILMAN A. DREW

From the University of Maine, Orono, Maine

THIRTEEN FIGURES

FOUR PLATES

This account, which deals with some of the sexual activities of the squid, is based upon observation made on specimens kept in glass sided aquaria at the Marine Biological Laboratory, Woods Hole, Mass. Specimens caught in the fish traps of the immediate vicinity may, by careful handling, be kept in aquaria in fairly good condition for a number of days. Such specimens occasionally copulate and eggs are sometimes laid.

There are two methods of copulation. By one method the spermatophores ejaculate their contents so the sperm reservoirs thrown from them are attached in a special depression on the inner side of the outer buccal membrane opposite the junction of the two ventral arms (figs. 8 and 10). They then slowly emit sperm, which are carried to and stored in, a special sperm receptacle that opens near this depression and is imbedded in the tissue of the outer buccal membrane (figs. 10 and 11). In this receptacle the sperm are mixed with a secretion and are not active. How long the sperm may be retained in the receptacle is not known, but there is some reason to think that they may be retained for at least some weeks. Females with eggs that can be fertilized may be found during the four months, June to late September, that I have worked at Woods Hole. Without exception every adult female that had not spawned had the sperm receptacle filled more or less completely with sperm, although in many cases the eggs were far from mature. This, together with the dormant condition of the sperm in the receptacle, and the fact that they seem to be poured out only during egg laying, point to a possible long retention. It is certain that the same female may have sperm reservoirs attached near this receptacle a number of times after it has been filled, and it is possible that the same sperm do not continue long in the receptacle. There seems, however, to be no evidence that they are discharged except during the period of egg laying.

The other method of copulation results in fastening the sperm reservoirs of the ejaculated spermatophores near the end of the oviduct (fig. 8, *s*) usually directly on its walls but sometimes on the mantle, gill or visceral mass. There is no special receptacle for the sperm from these sperm reservoirs. They escape into the water, becoming active as they escape, and pass out with the water through the funnel. The escape of the sperm is rather rapid but there are vast numbers in each reservoir, from which they are constantly poured like smoke from a chimney until the reservoir is empty. It is not known how long it takes to empty a reservoir but by keeping reservoirs from spermatophores that ejaculated in dishes of sea-water, and by examining reservoirs normally attached to the oviducts and buccal membranes of females, it seems probable that the sperm do not all escape for two or more days.

In aquaria I have seen rather more cases of copulation where the spermatophores are inserted into the mantle chamber than where the sperm reservoirs are attached to the buccal membrane. This may be because of the limited quarters in aquaria. In the larger floating tanks, in which specimens are sometimes kept before they are brought into the laboratory, the buccal membrane copulation seems proportionally more common than in aquaria, but even here the mantle chamber copulation seems to be rather more frequent.

The same individuals may copulate several times in the course of a few hours. In general the male is aggressive. The female may attempt to escape or she may be quite passive. Spermatophores seem to be inserted in the mantle chambers of only those females that are nearly ready to deposit their eggs. In the large number of trials made it was found that the eggs of these individuals were so nearly mature they could be artificially fertilized. Females that are nearly ready to deposit eggs have the nidamental glands considerably swollen and the accessory nidamental glands are highly colored with bright red. Wherever the spermatophores were inserted in the mantle chamber these glands were in this condition.

Before copulation both female and male are usually especially active and may be known as sexually excited animals by their peculiar movements. The female in swimming seems to be nervous or excited. She throws short but rapid puffs of water from the funnel, moves the tail fin very rapidly and, leaving the arms quite limp, spreads them apart and frequently throws them to one side. This gives the arms a jerky or trembling motion not shown in ordinary swimming. Except during the most rapid movements of the female, the male solemnly swims by her side, an inch or two away, but parallel, and with his head in the same direction. He frequently manipulates his arms, spreading them apart, commonly with the two dorsal arms elevated nearly or quite to a perpendicular position, and the third arms spread far to the sides (fig. 3). This position is not infrequently accompanied by localized activity of chromatophores. A spot may appear near the base of each third arm and a smaller spot on each second arm a little further from its base. These spots do not remain continuously while the male is in this attitude but suddenly appear with each increase of activity on the part of either the male or female. Occasionally blushing is quite general over the head and anterior end of the body and sometimes includes the whole body but the bodies of both animals generally remain colorless except for the special spots mentioned on the male. The attitude of the male, with elevated and spread arms, is not continuous but is assumed every few minutes, or in some cases seconds, and the arms may be brought into the usual position of a swimming animal for periods of many minutes.

Males do not all respond equally to the presence of sexually active females. Not uncommonly one male in an aquarium containing several males will follow the females around by the hour while the other males remain entirely inattentive. Usually when a male begins to show sexual activity he will follow a single female although other females that show similar activities are present in the aquarium. Occasionally he may change to another individual but he nearly always returns after a few minutes to the one to which he has been paying chief attention.

A few males have been observed that were so sexually excited they followed individuals around quite indiscriminately. Under such conditions I have upon three occasions seen a male catch another male and insert spermatophores into his mantle chamber. Two of the three instances were between the same individuals, the second performance being only a few minutes after the first. In each of these cases the male seized made great efforts to get away and finally to get hold of the male that was holding him but was unsuccessful. Upon killing the male that received the spermatophores, sperm reservoirs were found attached to the base of the left gill and to the adjacent visceral mass. Such exceptionally active males may copulate repeatedly with a single female. In a few cases this has been carried so far that the female has actually been killed. Even after the female has become entirely inactive and apparently dead the male may copulate with her several times. In one case, a male that had been several days without food, after copulating with a weakened female, retained his hold and killed her by eating a considerable hole through the mantle.

The male always uses the same arm for transferring the spermatophores. This arm, the left ventral, is not greatly modified, but a short distance from its tip some of the suckers, especially those in the row farthest from the midline of the body, and a ridge between the rows of suckers show modification (fig. 4, *h*). The peduncles of a dozen or more of the suckers of the outer row are considerably elongated and the sucking discs of a few, (six or eight) are greatly reduced in size or entirely absent. In both directions from these, the discs become increasingly normal until no modification is apparent. The suckers of the row toward the midline of the body are somewhat modified, the peduncles being somewhat shorter than those of the other suckers in the row, and the sucking discs somewhat smaller, but in none of the suckers of this row are the sucking discs entirely absent. A glandular plaited ridge extends lengthwise between the suckers of this region and gives off branches that join each of the peduncles. This ridge is highest and broadest opposite the suckers that are most modified and gradually disappears as the suckers become normal. At its highest point it has about the same elevation as the shortest modified suckers, which are adjacent. Sections of the modified portion of the arm show that the ridge and suckers mentioned are covered by a thick columnar epithelium that stains deeply. Many of these epithelial cells are filled with large rounded granules that stain with eosin. The cells that cover other portions of the arm are flattened or cubical, do not stain very deeply, and do not contain granules. It seems probable that the cells of the hectocotylized region secrete a substance that aids the arm in holding the spermatophores. The modified suckers probably make the bending and grasping necessary for the transfer of the spermatophores more easily accomplished.

The positions of the animals during copulation are rather hard to determine as the whole process generally does not occupy more than ten seconds and during this time the animals are usually swimming and the arms are changing positions, but by carefully focusing attention during different acts upon first one arm and then another, the positions and movements have been determined with some accuracy I think. Fig. 1 represents the positions of the animals while the arm of the male that bears the spermatophores is inserted into the mantle chamber of the female. This figure is the result of my conception of positions after having carefully observed copulation more than twenty times. Since drawing the figure many other observations have been made and the positions always seem to be essentially as given.

The male usually grasps the female while both are swimming. Occasonally the female may be resting on the bottom in the characteristic attitude, with the tips of the arms and the posterior end of the body touching and the head and funnel region somewhat elevated. If not swimming, she usually, when grasped, starts to swim, but in a few cases that I have observed she made no effort and left the bottom only as she was lifted or turned by the male. In every case the male attached from the left side of the female. He frequently swims close to her and brushes the tips of his arms along her head and mantle. Just before attaching, if both are swimming, he sinks slightly beneath her and grasps her body with his arms so that his right arms are all on the right side of her body and his left arms are all on her left side. The body of the male is seldom exactly ventral to the female but usually slightly toward the left side. Attachment is evidently made as nearly as possible in the required position but when the female darts ahead, as she frequently does, the male is likely to attach too far posteriorly. In such cases he does not let go his hold but crawls rapidly forward, arm over arm, until the right position is attained. Naturally the positions of the individual arms differ somewhat but in general the arrangement is reasonably well shown in fig. 1.

For about a second after his position is attained the arms seem busy in making firm attachments, then with a rapid sweep his left ventral arm is passed by the end of his funnel and is immediately inserted into the mantle chamber along the left side of her neck, near the funnel. During the act both animals are usually quite without color and the inserted arm of the male may be seen fairly distinctly inside the mantle chamber.

The movement of the arm past the funnel is rapid and only once have I actually seen the grasping of the spermatophores and their transference to the mantle chamber. In this case while watching squid in an aquarium that was placed so the squid were between me and a window, a male grasped a female that was resting on the bottom. The female, contrary to the usual custom, did not move. As the male had attached far back on the body, opportunity was given me to get into position for observation before the male could crawl forward. As the female made no attempt to get free, the male seemed far more deliberate than usual. Just before the arm was passed by the end of the funnel, the penis could be seen protruding into it. A number of spermatophores appeared in the opening of the funnel and were grasped by bending the tip of the arm around them. With a rapid sweep of the arm they were immediately inserted into the mantle chamber of the female where they were held about five or six seconds. The arm was then withdrawn and in about five or six seconds more the empty cases of the spermatophores passed out of the funnel of the female with a respiratory jet of water. These spermatophore cases were pretty closely attached to each other by having the tubes of their ejaculatory apparatus twisted together. They were recovered and found to be 41 in number. To the cluster were attached five sperm reservoirs. Examination of the female later showed that most of the other reservoirs were attached near the end of the oviduct. While the number of spermatophores used in an act of copulation varies greatly, the observations that have been made, indicate that this may be a little, but not much above the average.

The animals nearly always separate almost immediately after the arm is withdrawn. Beside the male which started to eat the female, a very few individuals have remained attached for from some seconds to nearly a minute after the arm has been withdrawn.

After copulation the female frequently seems considerably fatigued and may settle to the bottom and rest some minutes before becoming active again. I am rather inclined to think that this is due to her struggles, for when the female remained quiet, the apparent fatigue did not seem so marked. The male does not seem greatly affected, but is likely to continue to be very active for some time.

The copulation that leads to the filling of the sperm receptacle on the buccal membrane does not seem to be preceded by special movements. Although I have observed it several times the absence of preparatory movement has left me rather unprepared for the observations that must necessarily be made so quickly, for in this, as in the other form of copulation, the animals are seldom in contact more than ten seconds. In the cases I have observed my attention has been attracted by the sudden dart of one squid, the male, from one end of the aquarium directly at another, the female. Before the dart the squid face each other, and are separated by thirty centimeters or more. The movement was always exceedingly rapid and was probably due in each case to the expulsion of a single jet of water. The male seemed to reach the female before she had time to move much, although she has given me the impression of attempting to dodge as if frightened. The two animals become attached head to head with their arms intermingled, each grasping the other (fig. 2). Then as in the other method, the male sweeps his left ventral arm past the end of the funnel and grasps the bundle of spermatophores. These are immediately thrust between the ventral arms of the female and held there for a few seconds. The animals then separate and examination has shown fresh sperm reservoirs attached to the receiving depression on the buccal membrane of the female. The empty cases of the ejaculated spermatophores may be held between the arms several minutes but they are finally dropped. Here, as in the other method of copulation, only the sperm reservoirs are retained for any length of time.

The spermatophores begin to ejaculate immediately after leaving the penis and the whole process is completed in a very few seconds. Pulling the filament attached to the ejaculatory end of a spermatophore is all that is needed to start its ejaculation. As the ejaculatory end of the spermatophore leaves the penis last and, as the spermatophores in the penis and the spermatophoric sac are imbedded in a viscid secretion, there is every reason to believe that the pull given the spermatophores by the arm with which they are grasped, when this arm starts to transfer them from the penis to the mantle chamber or to the buccal membrane, is sufficient to start ejaculation. The arm carries the spermatophores into the position necessary for the attachment of the sperm reservoirs while they are ejaculating and holds them there until the ejaculation is complete and the reservoirs are attached.

The structure of the spermatophores and the mechanics of ejaculation which lead to the attachment of the reservoirs will be treated in another paper. It should, however, be understood that the spermatophores are never attached as such, but they ejaculate and the sperm reservoirs are attached. As the reservoirs are attached by cement carried inside the spermatophores and liberated by the ejaculation, they may be stuck anywhere.

The sperm slowly escape from these reservoirs and may then become free in the water, as when they are attached in the mantle chamber, or may be stored in a special receptacle, as when they are attached in the special depression on the outer buccal membrane. They are mixed with a viscid secretion in the reservoir and probably also before entering the reservoir, although I am not certain about the latter. The epithelium of the region is abundantly supplied with goblet cells which very possibly supply secretion for this purpose.

The depression in which the sperm reservoirs are mostly attached is supplied with a deeply staining columnar epithelium which is covered by a mass of rather hard material, evidently secreted by these cells, that shows distinct markings parallel with the surface of the epithelium (figs. 11 and 12). These markings seem to indicate that the material is secreted intermittently and thus is formed in layers. This material forms a suitable place for attachment of sperm reservoirs and probably serves no other purpose. Reservoirs are sometimes attached to other portions of the buccal membranes or to the tentacles but they are far more abundant in the depression than anywhere else. The sperm that escape from the reservoirs that are not attached in this depression probably do not find their way into the sperm receptacle.

The sperm receptacle has the shape of a compound alveolar gland (fig. 11). It is imbedded in the outer buccal membrane and opens on the inner surface of this membrane at a point opposite the junction of the two ventral arms. Simple cubical epithelium lines the deeper alveoli of the receptacle, and cubical epithelium with many goblet cells the portion nearer the opening. Some, but not many, cilia have been seen on these cells. The killing fluids used may not have preserved them, for the tails of sperm in the reservoirs are not often individually visible in the sections. With the exception of the tails of the sperm and the possible cilia on the cells the material gives evidence of good preservation. A layer of muscle fibers surrounds the receptacle as a whole and bundles of fibers run between and around the individual alveoli.

It was not determined whether the sperm are active in the interval between their discharge from the reservoirs and their entrance into the receptacle or not. That they are not active while stored in the receptacle is shown by opening filled receptacles on dry slides. The sperm are invariably quiet, but immediately become active when sea-water is added. In specimens killed soon after copulation, sections show the sperm entering the receptacle in narrow streams and not spread out as one might expect them to be if the sperm were active (fig. 11). It was not possible to remove all the sea-water from living specimens in which the receptacles were being filled without causing disturbances in the vicinity of the reservoirs and that made it impossible to determine the normal condition of the sperm in transit from one to the other. In the sections that show sperm entering the reservoir the tails all point in the same direction, as would be the case if they were not swimming actively but were being moved by an outside force. The heads go first and the tails all trail behind. Swimming sperm usually move in all directions but there may be some directive cause that would account for their positions even if they are stored through their own activities.

As previously stated, a female that is nearly ready to deposit her eggs can be told by her peculiar nervous movements and the way she manipulates her arms. Frequently the borders of the accessory nidamental glands, which are very red at this time, may be seen through the semi-transparent mantle and thus form a further indication that the eggs are nearly ready to be deposited. The nidamental and oviducal glands of such an animal are always somewhat, and frequently greatly, enlarged. Immediately after the eggs have been deposited these glands, while still large, are soft and flabby.

As is well known the squid deposits her eggs imbedded in strings of a jelly-like substance which vary in size with the size of the animal depositing them but which probably average about 8 mm. in diameter and 90 mm. long. The jelly consists of an inner mass that surrounds the eggs and a thick, rather tough but still jelly-like sheath that forms the outer covering. The inner jelly is secreted inside the oviduct by the oviducal glands. The outer jelly is secreted by the nidamental glands and is apparently moulded into shape as it passes through the funnel. The accessory nidamental glands, which lie just in front of the anterior ends of the nidamental glands and open by wide openings near the narrow openings of these glands, are very active during this period and secrete a viscid material. What the special function of this secretion is has not been determined. It would seem from position and activity that the secretions from both sets of glands must be mixed as they are poured out.

Until recently eggs have not commonly been deposited in aquaria at Woods Hole. This may be due to the way the animals have been handled. Squid will not stand rough handling, either in capture or transportation, and live well in aquaria afterward. When captured in fish traps, quickly and carefully transferred to live cars where they are supplied with an abundance of water, and transported to the aquaria with as little excitement and as good water as possible, they may be kept several days in pretty good condition, but they wear themselves out by constantly bumping against the walls of the aquaria and are not vigorous many days. During each of the months I have worked at Woods Hole, June to late September, specimens have been obtained that have deposited eggs in aquaria. During the first three months specimens ready to deposit eggs are rather easy to get. In September only a small proportion of those captured still contained eggs.

Eggs are somewhat more frequently deposited in aquaria at night than during the day, but this may be due to the frequent if not nearly continuous disturbance to which they are subjected during the day in a laboratory where many people are working. The usual number of strings deposited by a female in what would seem to be a continuous laying period ranged from one to six. These strings were commonly delivered from fifteen to forty minutes apart, the time between any two strings being quite variable in an individual. One specimen, however, deposited twenty-three strings in an hour and thirty-five minutes. These were laid during a comparatively dark day when the laboratory was quiet. Possibly the small number deposited by other females was due to disturbance.

The end of the egg string begins to protrude from the end of the funnel while the female rests upon the bottom in the attitude habitually assumed by resting squid (fig. 5). When from one to two centimeters of the egg string protrudes from the funnel, the female leaves the bottom and begins to swim slowly backward. This swimming is apparently due both to movements of the tail fin and to small jets of water forced from the funnel along the sides of the egg string. The jets of water cause the egg string to be protruded gradually. The protruding end is now caught by the ends of the two dorsal arms, which are bent ventrally between the other arms for this purpose (fig. 6), and as the string is ejected from the funnel, it is drawn between the circlet of arms. It usually takes from half a minute to a minute for the egg string to pass through the funnel and to disappear between the arms. It is then held between the arms about two minutes or sometimes a little longer. While the string is held between the arms it is completely enclosed by them and their free ends keep twisting around each other. In this position they form a cone with the apex at the ends of the arms (fig. 7). At other times the arms are held so they form a dorso-ventrally flattened expansion that serves somewhat as a rudder or anterior fin. The arms while enclosing the eggs are never entirely still but move slightly upon each other and are probably busy in moving the string about. While the string is thus held the animal slowly swims back and forth, never rapidly but continuously.

Toward the end of the period during which the string of eggs is held, the animal shows an increasing tendency to turn the body into a nearly perpendicular position to bring and keep the tips of the arms in contact with the bottom (left animal in fig. 9). With the arms held quite rigid and the tail fin moving rapidly she goes bounding along on the tips of her arms, dorsal side foremost, with a movement somewhat similar to the bounces that may be obtained by pushing a lead pencil, held by one extremity and slightly inclined from the perpendicular, over a table. This action is generally repeated several times. She occasionally catches hold of objects with her suckers, finally catches some object firmly, draws down into close contact with it for two or three seconds (right animal in fig. 9) and, when she releases her hold, leaves the string of eggs fastened to the object she had laid hold of. At this time the jelly of the string is soft and sticky. It hardens quite rapidly and soon will not stick to objects, but at this time it adheres readily. The position of the string when taken between the arms indicates that the string is finally stuck by the end that first leaves the funnel.

After sticking a string of eggs the female rests upon the bottom some minutes before another string makes its appearance. She usually selects some protruding object like a stone, shell, or water pipe upon which to stick the egg strings. Having stuck one string she usually, but not always, returns to the same place to stick later strings. If strings are present when a female begins to deposit she usually attaches to these strings, or to nearby objects. This no doubt accounts for the very large clusters, with strings containing eggs in various stages of development, that are sometimes found. Upon several occasions clusters in fish-traps and live-cars have been found that would not go into an ordinary ten-quart pail. Such clusters are of course formed by many females.

It is evident that the eggs may be fertilized in the oviduct, in the mantle chamber, or between the arms. Examination of the contents of the oviduct have in no case given evidence of sperm. Eggs taken from the oviduct may easily be fertilized by placing them in sea-water containing sperm, but in no case did eggs taken from the oviduct show evidence of fertilization although many sperm reservoirs that were giving off active sperm were attached to the walls of the oviduct and to surrounding organs.

There can be no doubt, however, that eggs may be and are fertilized in the mantle chamber and also between the arms. That the eggs may be fertilized in the mantle chamber is indicated by reason rather than by obervation. When sperm reservoirs are attached in the mantle chamber the sperm are constantly liberated in the water in this chamber as long as the supply lasts. The eggs upon leaving the oviduct also pass into the mantle chamber and, as before stated, when eggs and sperm are mixed in sea-water, fertilization results.

That fertilization may be delayed until the egg string is formed and held between the arms is indicated by observations made on the female already mentioned that deposited twenty-three strings. She was in a rather large aquarium with a number of other squid. Copulation had occurred several times but this particular squid, which had been under observation some hours, had not been seen to copulate. Dissection later showed that there were no sperm reservoirs attached in her mantle chamber. Because of disturbance she upon six occasions failed to get the egg string between her arms. When she reached for the string with her dorsal arms she was each time disturbed so she dropped the string and ejected it directly into the water. Four of these strings were recovered as quickly as possible after they were dropped, and placed in dishes of fresh sea-water where the proportion of fertilized eggs could be determined. From 40 to 50 per cent of the eggs in the strings developed. More than 99 per cent of the eggs in strings that had been held between the arms and then placed in similar dishes developed. As already mentioned there had been copulation among other squid in the aquarium and as the reservoirs were attached in the mantle chambers there must have been many free sperm in the water of the aquarium. It seems probable that enough of these sperm reached the strings that were dropped, before they could be removed from the aquarium, to fertilize a portion of the eggs. Microscopic examination of these strings immediately after they were dropped revealed very few sperm, but the strings that were held between the arms were swarming with them. Sperm were able to penetrate and move actively about in the soft jelly of a recently formed string, but the jelly soon hardened so fresh sperm brought in contact with it were not able to work their way in.

A curious bit of habit reflex was exhibited by this squid each time she dropped a string of eggs. Immediately after the disturbance she took the attitude she would normally have taken had the egg string been successfully lodged between the arms. The arms were held in the form of a cone, the tips were twisted together and she passed on through each of the succeeding phases even to drawing down tight against an object as if to attach the egg string that had never been between the arms. After this she rested until the next string was formed, but she never interrupted the orderly sequence of her activities because she had accidentally lost a string of eggs.

The methods of copulation of cephalopods have attracted the attention of observers from very early times but the act of copulation has not been actually seen for many species and where observations have been made they have for the most part been incomplete. Aristotle makes several statements regarding the breeding habits of cephalopods. It is quite possible that he saw something of the act of copulation for some species, but his statements are hard to follow and are evidently inaccurate. The most important statements are here quoted to show the curious medley of facts and fiction. In chapter 5, book 5, he says:

1. All the malacia, as the polypus, sepia and teuthis, approach each other in the same manner, for they are united mouth to mouth: the tentacula of one sex being adapted to those of the other; for when the polypus has fixed the part called the head upon the ground, it extends its tentacula which the other adapts to the expansion of its tentacula, and they make their acetabula answer together. And some persons say that the male has an organ like a penis in that one of its tentacula which contains the two largest acetabula. This organ is sinewy, as far as the middle of the tentaculum, and they say it is all inserted into the nostril of the female.

2. The sepia and loligo swim about coiled together in this way, and with their mouths and tentacula united, they swim in contrary directions to each other. They adapt the organ called the nostril of the male to the similar organ in the female; and the one swims forwards, and the other backwards. The ova of the female are produced in the part called the physeter, by means of which some persons say that they copulate.

Again in chapter 10, book 5, he says:

1. The malacia breed in the spring, and first of all the marine sepia, though this one breeds at all seasons. It produces its ova in fifteen days. When the ova are extruded, the male follows, and ejects his ink upon them when they become hard. They go about in pairs. The male is more variegated than the female, and blacker on the back. The sexes of the polypus unite in the winter, the young are produced in the spring, when these creatures conceal themselves for two months. It produces an ovum like long hair, similar to the fruit of the white poplar. The fecund-

ity of this animal is very great, for a great mumber of young are produced from its ova. The male differs from the female in having a longer head, and the part of the tentaculum which the fishermen call the penis is white. It incubates upon the ova it produces, so that it becomes out of condition, and is not sought after at this season.

Part of these statements, such as "The sepia and loligo swim about coiled together in this way, and with their mouths and tentacula united, they swim in contrary directions to each other" would seem to be based upon such observations as could be made from above but the further statement that they adapt their nostrils (funnels) together, probably indicates the ease with which observation and supposition can be mixed. It is not necessary further to analyze Aristotle's statements. No doubt much was based upon fishermen's stories but he evidently did study the anatomy and habits of these animals and recognized the probability that one of the arms of the male is used in copulation.

While the modified arm of the male thus early received attention, the true hectocotylus that separates entirely from the male and attaches itself in the mantle chamber of the female escaped notice for many centuries. To quote from the Cambridge Natural History:

The typical hectocotylus seems to have entirely escaped notice until early in the present (last) century, when both Delle Chiaje and Cuvier described it, as detected within the female, as a *parasite,* the latter under the name of Hectocotylus octopodis. Kölliker, in 1845–49 regarded the Hectocotylus of *Tremoctopus* as the entire male animal, and went so far as to discern in it an intestine, heart, and reproductive system. It was not until 1851 that the investigation of Vérany and Filippi confirmed a suggestion of Dujardin, while H. Müller in 1853 completed the discovery by describing the entire male as Argonauta.

While nearly all male cephalopods show some modification of one or more arms, the only ones that have been reported with detachable arms are Argonauta, Ocythöe, and Tremoctopus.

Extended studies have been made on the modification of the arms of cephalopods, and there have been a few observations upon the functional activities of these arms, but most of the observations have consisted in finding sperm reservoirs recently attached to various portions of females.

In 1869 Lafont described copulation in Sepia. A translation of that portion that deals with the act itself is as follows:

> In copulation the male and female precipitate themselves upon one another, hold together by their arms which are twined together, and remain thus, mouth to mouth, for a variable time, which may last for two or three minutes. This act is followed in the female by a state of very marked general prostration, while in the case of the male the general excitation is greatly prolonged and for a considerable time it keeps the splendid appearance these animals show as the result of the accomplishment of the function of reproduction.

He supposed that while the animals were attached by their arms, head to head, the male ejected a packet of spermatophores, which ejaculated while in his mantle chamber and the sperm reservoirs were then thrown from the funnel of the male into the branchial chamber of the female with the current of water entering her branchial chamber.

Sepia, like Loligo, has a receptacle for the storage of spermatozoa in the buccal membrane, and the position observed by Lafont of animals attached head to head was doubtless a true position of copulation, but it seems probable that the spermatophores were not disposed of in the way suggested, but were transferred to the buccal membrane by one of the arms of the male. Lafont found sperm reservoirs attached in the mantle chamber of the females near the mouths of the oviducts, so it seems probable that in this form, as in Loligo pealii, both methods of copulation occur.

Racovitza (1894, a) observed copulation in Sepiola. The male seized the female, turned it over and inserted his first pair of arms into the mantle chamber. Copulation lasted eight minutes during which the female struggled to free herself. He speaks of the spermatophores being fixed on the folds of a large pocket situated on the left side of the pallial cavity of the female. These ejaculate and the freed reservoirs deliver their sperm into the pocket which in turn ejects them (from his description I take it they are not stored up in this pocket as in the receptacle on the buccal membrane of a squid) into the pallial cavity where they are supposed to meet the eggs as they are laid.

The most complete account of copulation that I have seen for any cephalopod was given by Racovitza in 1894 (b) for Octopus vulgaris. He observed copulation in an aquarium and gives a figure showing the positions of the animals. The copulation differs markedly from that of Loligo, as might be expected, for Octopus has a hectocotylized arm that is much more differentiated than that of Loligo. The animals were some distance apart in the aquarium. The male reached over with the hectocotylized arm, which for this species is the third on the right side and, after caressing the female with its tip, introduced its end into her mantle chamber by the side of the funnel. Here it remained for something more than an hour. During this time the female remained quiet, except for certain spasmodic movements, while the male showed only slight movements of the hectocotylized arm which were supposed to be associated with the movements of spermatophores down the longitudinal groove of this arm. Although it was not possible actually to see the spermatophores in transit, examination of the female after copulation showed numbers of the sperm reservoirs, derived from the ejaculated spermatophores, within the oviducts.

Evidently there are at least three methods of copulation practiced by cephalopods. A method of caducous hectocotylism in which the charged hectocotyl is liberated in the mantle chamber of the female; a method in which the arm does not liberate any special portion but is so modified that it can transfer spermatophores by a mechanism within itself to the region of the oviduct of the female; and finally a slight modification of the arm that simply enables it to grasp the spermatophores which are then transferred directly to the female by moving the arm. Where the latter method is employed there may be two kinds of copulation, as in Loligo pealii.

Racovitza, (1894, c) in commenting on the copulation of Rossia believes that, although special receptacles are found outside the mantle chamber of this species, they cannot be considered as normally functional. He seems led to this conclusion by finding sperm reservoirs attached to various portions of the bodies of the animals as well as in the immediate neighborhood of the mouths of the oviducts. It would seem more likely in the light of the observations here recorded for Loligo, that a copulation that leads to the filling of these receptacles is normal and that the sperm so stored may be used in fertilizing the eggs.

It is certainly hard to conceive by what steps a complicated method of transferring sperm that has led to the formation of a hectocotylized arm and complicated spermatophores might be perfected. The modification of different arms for copulation by different cephalopods further increases the difficulty in understanding the history of hectocotylism as a whole.

While evidence that bears directly upon the history of the hectocotylism seems to be lacking, such complications are so frequently considered to be impossible to explain by known evolutionary factors that it may be well at least to consider the great difficulties presented by such structures. It must not be supposed that in so doing I put myself in the position of defending a thesis. This would be too much like the methods employed by many of the Greek philosophers who needed little or no basis of fact upon which to build. My only reason for considering the matter here is to show that, with all the difficulties, the condition of hectocotylism among modern cephalopods cannot be considered beyond the possible range of evolutionary factors.

Among the Dibranchiata the arms that show hectocotylism are the first, the third and the fourth on both sides of the body. Sometimes more than a single one is affected. In such cases the modified arms may be symmetrically placed on the two sides of the body, or they may be adjacent arms on the same side of the body. Steenstrup attempted to base the classification of cephalopods upon their hectocotylized arms but Brock and Hoyle have shown that forms whose general body structure would seem to indicate relationship, do not always have homologous arms modified.

While the arm is usually constantly on one side for all members of a genus, unless both sides are modified as not infrequently happens, a genus whose general body structure indicates near relationship may have the similar arm of the other side modified. The position of the arm on the right or left side of the body is not generally considered very significant. The somewhat frequent occurrence of genera showing hectocotylism of arms symmetrically placed on the two sides of the body may indicate a primitive paired condition that has been replaced among the majority of existing cephalopod genera, by specializing on one side and dropping out on the other. Whether this accounts for the condition or not, the shifting of a modification from one side of the body to the other, sometimes involving modifications of other body structures and sometimes apparently not, is not uncommon among animals, and even if not easily explained, evidently has no very great phylogenetic significance. Shifting in series is not so common and when we find in the same family, genera with the fourth and others with the first arm hectocotylized it becomes difficult to imagine ancestral conditions that made this posisble.

Wherever known, male cephalopods use one or more of the arms to transfer sperm to the female. Copulation has not been described for many of the species but the presence of more or less modified arms in more than half the recognized families may be taken as an indication that either these animals or their ancestors used their arms in copulation.

If the spadix of Nautilus is used in copulation we have a possible indication that a number of arms may have been employed in the transfer of sperm by primitive cephalopods. It is of course possible that all the arms were used for this purpose and that the present diversity can be accounted for by the specialization of one or the other of the arms involved in this primitive condition. This, however, does not seem reasonable when the diversity within the limits of a single family is considered.

The arm that is used, and the way in which it is used, is associated with the character of the spermatophores and the position of their final discharge. The Octopoda show the greatest structural modification in their hectocotylized arms. While two of the families of this group give no evidence of hectocotylism, none of the genera of the remaining families are known to be free from it, and wherever found it is always the third arm that is involved. Sometimes this arm is on the right and sometimes it is on the left side. In three genera it is known to be caducous and in a fourth (Alloposus) it is supposed to be. In the remaining genera in which the hectocotylized arm has been studied, the modifications, while not resulting in the actual separation of the arms, are of an extensive nature. In Octopus, for instance, they involve not only changes in size, form, and the condition of suckers, but a special groove is present through which the spe matophores are supposed to be carried from the base, presumably from the penis to the tip. The tip in turn is modified so it is supposed to function in placing the spermatophores in position for ejaculation.

The Decapoda do not show such extensively modified hectocotylized arms. The changes are here chiefly confined to some of the suckers and their immediate vicinity. In Loligo this modification apparently serves to aid the arm in grasping the spermatophores, which are then transferred by the movement of the arm. While the actual grasping of the spermatophores has not been previously observed, there can be little doubt that other forms of the Decapoda use the arms in a similar manner. Where copulation has been observed the movements of the arms indicate that they are used in the transfer, and the positions of the sperm reservoirs that have been found attached to the females indicate that some arm must have functioned in getting them into position. As there is no special transferring mechanism, this must have been accomplished by the free movements of the arms.

Where structural modification is slight and the placing o the spermatophores is due to dexterity, there is less difficulty in understanding how the function may be shifted from one arm to another in response to changes in the position of the attachment of the reservoirs on the female, than would be the case were great structural changes involved. It would be much more difficult to understand how there could be a shifting in series of arms as highly modified as those of the Octopoda, where only the modified arm could possibly perform the function.

It must not be understood that habit formation requiring such dexterity is considered easier to originate than modification in structure that will perform similar acts. When, however, the habit and dexterity have been acquired, it is not inconceivable that they might be shifted to another closely similar appendage if the position of this appendage becomes more suitable for the purpose. The modification is so slight in the arms of most of the Decapoda, and the modification varies so greatly in the different genera, that it may have been functionally acquired in each case. So far as can be seen it would be mechanically quite possible for a squid to use an unmodified arm, instead of the one that shows the modification, for the transfer of the spermatophores. The spermatophores might not be so tightly or compactly held but the normal suckers would hardly seem to interfere greatly in the performance of the function.

There is still another question involved. Is there any genetic relation between these two methods of transfer and if there be, which, if either, most probably came first?

A special method of copulation that requires the use of arms and complicated spermatophores is not found among animals often enough to make it at all probable that it has arisen in this group more than once, so we can hardly doubt that the two methods are genetically related.

At first sight the squid's method of grasping the spermatophores and transferring them directly might be considered the simpler process, but there is some reason to doubt that this method was at the beginning of the series. While it would be hazardous to say that Octopoda were the ancestors of Decapoda, there is much reason to believe that the ancestors of the latter lived upon the bottom and were far less active than the modern animals. Such animals would not seem to be so well adapted for the transfer of spermatophores by dexterous movements as the more active, free-swimming forms. It is at least certainly true among modern cephalopods that those that show great modifications in the structure of the hectocotylized arms are found entirely among the less active bottom forms. If the method of transferring sperm by means of the arms originated before the Decapoda became free-swimming animals, and this seems the only explanation of its prevalence among both Decapoda and Octopoda in modern times, it would seem that structural modification probably came early.

Possibly this modification was based upon the use of one or more arms as guides for the transfer of the sperm. It is possible that having first used the arms as guides, structural modifications and dexterous movements were developed as divergent methods. If the two methods form a linear series, there is some reason to think structural modifications came first. It would seem much easier to explain modifications that lead to the change in the structure of appendages for the transfer of spermatozoa, as the grooved hectocotylized arm of Octopus or the modified abdominal appendages of certain Crustacea, than to explain a sudden change that would result in a practically unmodified arm functioning by grasping spermatophores of a very specialized kind, transferring them quickly and accurately to the required position and holding them there until they have had ample time to ejaculate and fix their contents. It seems more reasonable to suppose that an arm modified as a machine to perform this process, with its tip serving to place the spermatophores in position, might in time acquire the necessary dexterity and then lose the modifications previously acquired, than to look at this as the beginning of the series. Again we find that in such cases as the squid, where the arm is little modified but very dexterous, there is a special receptacle at some distance from the opening of the oviduct that is norma ly filled with sperm during the breeding season. This would certainly seem to be a comparatively recently acquired receptacle, so the copulation leading to its being filled would also be considered comparatively recent. That this receptacle is concerned in the fertilization of the eggs is shown by observations made while the eggs were being laid.

With no personal knowledge of the breeding habits of other cephalopods than the squid, it would seem more reasonable to consider the method of using the detachable hectocotyl of such forms as Tremoctopus as one extreme, the method used by Loligo in grasping spermatophores and transferring them directly as another extreme and the condition shown by Octopus as the modern greatly specialized product of a modification such as early cephalopods probably developed. This would mean that the detachable hectocotyl is an extreme specialization in structure and that the modification shown by the squid represents possibly a degeneration in structure but a remarkable specialization in habit.

Why a form should have two methods of copulation is not at all clear. Certainly the introduction of the spermatophores into the mantle chamber to a position near the oviduct is to be considered more primitive than their being placed in a position to fill a receptacle outside of the mantle chamber, but why mantle chamber copulation should be retained after the receptacle has been perfected is not clear. That mantle chamber copulation is not absolutely necessary for the fertilization of the eggs I think is proved by my observations; that it is common is certain. That the sperm receptacle is an improvement over the free attachment of the sperm reservoirs in the mantle chamber is evident from the longer possible retention of the sperm in the receptacle. In a limited period after the sperm reservoirs are freed from the spermatophores, as when deposited in the mantle chamber, the sperm all escape and are wasted unless oviposition takes place in the meantime.

SUMMARY

Squid have two methods of copulation. By one method sperm reservoirs are attached in the mantle chamber on or near the oviduct and immediately begin to discharge their contents freely in the water. By the other method sperm reservoirs are attached to the outer buccal membrane and the sperm become stored in a special receptacle in the membrane, which is placed opposite the junction of the two ventral arms and opens on its inner surface.

The left ventral arm of the male is always used in transferring the spermatophores, which are grasped by the arm and transferred by its free movement. Ejaculation of the spermatophores is evidently started by the pull given their filaments when the arm starts to transfer them from the penis to the mantle chamber or buccal membrane. The transfer requires rapidity and dexterity and the spermatophores are held in position until ejaculation is complete and their sperm reservoirs are fastened. As many as forty spermatophores may be transferred at a time.

The egg strings are composed of two kinds of jelly. One kind is supplied by the oviducal gland and the other by the nidamental and probably accessory nidamental glands. The string is apparently molded into shape by passing through the funnel. The jelly is at first soft and sticky but soon becomes tough and loses most of its stickiness.

From the funnel the egg string is drawn between the circlet of arms, where it is held two or more minutes. In sticking the string the female grasps some object with her arms and draws down tight so the string is evidently crowded against it. When she releases her hold the string is left sticking to the object.

Fertilization evidently does not take place inside the oviduct. It doubtless may take place in the mantle chamber when sperm reservoirs are present there, and is known to take place while the egg string is held between the arms. The sperm are liberated from the receptacle while the eggs are between the arms.

Notwithstanding complications, the conditions of hectocotylism shown by cephalopods need not be considered beyond the influence of factors of evolution.

LITERATURE CITED

The cephalopod literature is very extensive. Only those papers directly referred to are here given.

Aristotle History of animals. Trans. by Richard Cresswell. 1891.

Brock, J. 1882 Anat. u. Syst. d. Cephalopoden. Z. f. wiss. Zool. 36.
1884 Männchen d. Sepioloidea lineata. Z. f. wiss. Zool. 40.

Hoyle, W. E. 1907 Presidential address of Zoölogical Section. Rept. Brit. Ass. Adv. Sci.

Lafont, M. A. 1869 Observations sur la fécundation des Mollusques Cephalopods der Golfe de Gascogne. Ann. des Sci. Nat. (5) 11.

Racovitza, Emile. G. 1894a Sur l'accouplement des quelques Cephalopods Sepiola rondeletii (Leach), Rossia macrosoma (d. Ch.) et Octopus vulgaris (Lam.). Comp. Rend. l'Acad. des Sci. 118.
1894b Notes de Biologie. I. Accouplement et Fécondation chez l'Octopus vulgaris Lam. Arch. d. Zool. Exper. et Gen. (3) 2.
1894c Notes de Biologie. III. Moeurs et Reproduction de la Rossia macrosima (D. Ch.). Arch. d. Zool. Expér. et Gen. (3) 2.

Steenstrup, J. J. S. 1856–57 Hectocotyl. hos Octopodstægterne. Vid. Selsk. Skr. (5) 4, Translated Ann. N. H. (2) 20.
1881 Sepiadarium og Idiosepius. Vid. Selsk. Skr. (6) L.
1887 Notæ Teuthologicæ 7. Overs. Vid. Selsk. Forh.

EXPLANATION OF FIGURES

All of the figures that represent the attitudes of squid were drawn from memory after repeated observations. While each figure is thus really a composite, and must represent impressions received rather than the actual positions of particular individuals, much care has been given to the preparation of the figures and it is believed that the general attitudes are reasonably well represented. Sexually mature squid are usually as much as 15 cm. and may exceed 40 cm. in length.

ABBREVIATIONS

bmi, inner buccal membrane
bmo, outer buccal membrane
d, depression in which sperm reservoirs are attached
g, gill
h, modified (hectocotylized) portion of arm
j, jaws
n, nidamental gland
na, accessory nidamental gland
o, oviduct
r, rectum
s, sperm reservoirs (ejaculated from spermatophores)
sr, sperm receptacle
sro, opening of sperm receptacle

PLATE 1

EXPLANATION OF FIGURES

1 Copulating squid showing the positions taken by the animals when the spermatophores are inserted into the mantle chamber. The figure shows the animals during the period the arm of the male is inserted in the mantle chamber of the female. Drawn from memory after many observations.

2 Copulating squid showing the positions of the animals when the spermatophores are placed so that their reservoirs become attached to the outer buccal membrane. The figure shows the male in the act of grasping the spermatophores with the tip of his arm as they are ejected through the funnel. Drawn from memory after many observations.

3 A common attitude of a sexually excited male. The arms are not kept rigidly in a set position, but are frequently spread as shown in the illustration and held thus for from a few seconds to a minute or more at a time. The drawing is based upon sketches made of active animals.

4 Photograph of the two ventral arms of a male squid, showing the slight modification (*h*) consisting of enlarged peduncles, reduced sucking discs and a ridge between the suckers, toward the tip of the left arm. The wrinkles on the arms are due to shrinkage. A bit of the outer buccal membrane shows between the arms. The arms from which the photograph was made are 9½ cm. long.

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PLATE 2

EXPLANATION OF FIGURES

5 A female at rest with the egg string beginning to protrude. Drawn from memory and hurried sketches after many observations.

6 A female after she starts to swim, reaching for the egg string with her dorsal arms. With these arms she draws the string between the circle of arms as it is ejected from the funnel. Drawn from memory after many observations.

7 A swimming female, showing the positions of the arms while they surround the egg string. They are held in this position, with the tips somewhat twisted together, for two or three minutes. While the arms closely surround the egg string they show slight individual movements that may be of service in moving the egg string so sperm will be more evenly distributed over it. Drawn from memory after many observations.

8 A female squid with the mantle cut and spread open and the arms separated to show the position of attached sperm reservoirs (*s*) on the oviduct (*o*) and the sperm receptacle (*sr*) in the outer buccal membrane.

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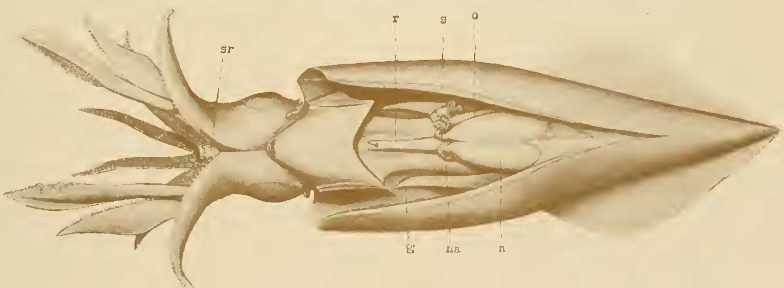

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PLATE 3

EXPLANATION OF FIGURE

9 The specimen on the left side shows a female in the position she assumes as she bounces over the bottom on the tips of her arms just previous to selecting a place for sticking the egg string. The specimen on the right side shows the position of a female during the act of sticking an egg string to a rock. Only a few seconds are required to stick the string. The positions of the animals are drawn from memory after many observations.

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Drew, del.

PLATE 4

EXPLANATION OF FIGURES

10 Jaws and buccal membrane of a female squid, with the outer membrane (*bmo*) pulled ventrally to expose the sperm receptacle (the opening of which is shown at *sro*) and the adjacent depression (*d*). Several sperm reservoirs (*s*), ejaculated from spermatophores, are shown attached in the depression. Magnified about 7 diameters.

11 Section of the outer buccal membrane taken through the sperm receptacle (*sr*). This was taken from an animal shortly after the sperm reservoirs (*s*) had been attached and shows sperm in transit from reservoirs to receptacle. Magnified about 22 diameters.

12 Section through the epithelium and secretion lining the depression in which sperm reservoirs are attached. Magnified about 300 diameters.

13 Section through an alveolus of a sperm receptacle. The clear spaces in the epithelium are goblet cells. Traces of the flagella on the sperm and possibly cilia on some of the epithelial cells were visible but they were not definite enough to be put in the drawing. Magnified about 300 diameters.

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